Phytochem Rev (2010) 9:1–17
DOI 10.1007/s11101-009-9155-3

The cinnamate/monolignol pathway

Toshiaki Umezawa

Received: 17 March 2009 / Accepted: 13 October 2009 / Published online: 17 November 2009

Abstract The cinnamate/monolignol pathway provides precursors for various phenylpropanoid compounds including lignins, lignans, neolignans, *p*-hydroxycinnamate esters, coumarins, suberins, flavonoids, stilbenes and so on. Therefore, the pathway plays the central role in plant secondary metabolism. During the last decade, significant advances have been made in understanding the major routes and transcriptional control mechanisms of the pathway. In this review, the major routes and the transcriptional control are outlined in relation to lignification.

Keywords Lignin · Biosynthesis · Transcription factor · Monolignol · Phenylpropanoid · Biosynthetic pathway

Introduction

Lignin is one of the major components of the secondary cell wall of vascular plants, and fills the spaces between cell wall polysaccharides. It confers mechanical strength and imperviousness to the cell wall (Boerjan et al. 2003). Therefore, lignin biosynthesis is closely related to the evolution of land plants. Lignin has several properties that present obstacles to chemical pulping, forage digestion, and conversion of plant cell wall polysaccharides into biofuels. For these processes, it would be beneficial for plant materials to either have less lignin, or to have lignin that is easier to remove. For these reasons, lignin biosynthesis is an area of great interest (Dixon and Reddy 2003; Chiang 2006; Vanholme et al. 2008; Weng et al. 2008a).

Lignin is biosynthesized via oxidative coupling of *p*-hydroxycinnamyl alcohols (monolignols) and related compounds that are formed in the cinnamate/monolignol pathway. Recently, the pathway has been extensively revised, and the pathway's control mechanisms are being studied intensively (Chiang 2006; Vanholme et al. 2008; Weng et al. 2008a). In this review, the major routes and the transcriptional control of the cinnamate/monolignol pathway are outlined briefly in relation to lignification.

Earlier views of the cinnamate/monolignol pathway

Monolignols are biosynthesized from cinnamic acids in the cinnamate/monolignol pathway (Fig. 1). This pathway supplies precursors for various phenylpropanoid

T. Umezawa (✉)
Research Institute for Sustainable Humanosphere, Institute of Sustainability Science, Kyoto University, Uji, Kyoto 611-0011, Japan
e-mail: tumezawa@rish.kyoto-u.ac.jp

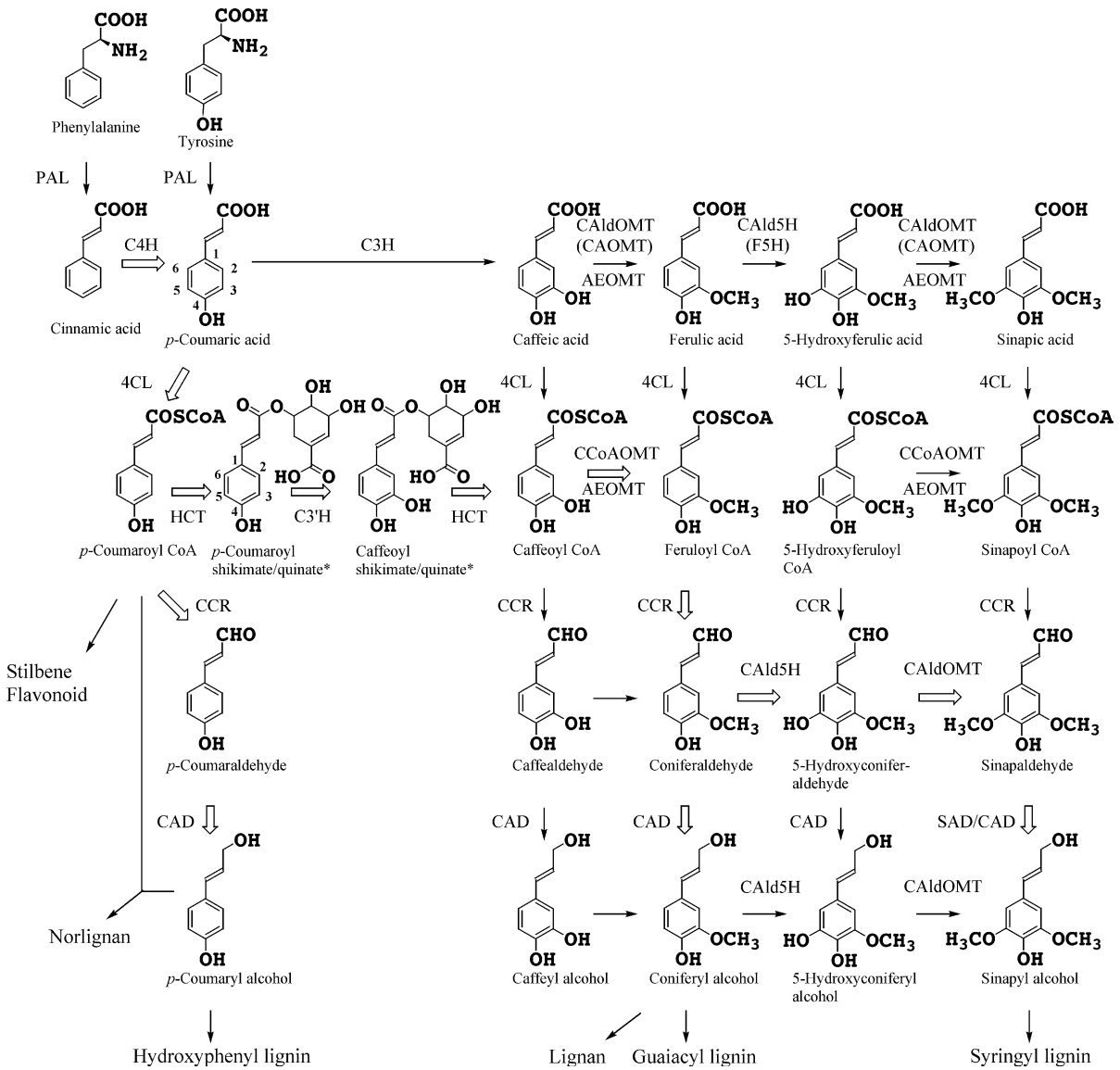


Fig. 1 The cinnamate/monolignol pathway. *Thick open arrow* represents the major routes for lignin biosynthesis. C4H, cinnamate 4-hydroxylase; C3H, *p*-coumarate 3-hydroxylase; CAldOMT, 5-hydroxyconiferaldehyde *O*-methyltransferase; 4CL, 4-hydroxycinnamate CoA ligase; HCT, hydroxycinnamoyl CoA: shikimate/quinate hydroxycinnamoyl transferase; CCoAOMT, caffeoyl CoA *O*-methyltransferase; CCR, cinnamoyl CoA reductase; CAld5H, coniferaldehyde 5-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; SAD, sinapyl alcohol dehydrogenase; F5H, ferulate 5-hydroxylase; CAOMT, caffeic acid *O*-methyltransferase; AEOMT, hydroxycinnamic acid/hydroxycinnamoyl CoA esters *O*-methyltransferase; C3'H, *p*-coumaroyl shikimate/quinate 3-hydroxylase. *Asterisks*: Only the structure of shikimate ester is shown

compounds such as lignins, lignans, neolignans, norlignans, *p*-hydroxycinnamate esters, coumarins, suberins, flavonoids, and stilbenes (Ishii 1997; Umezawa 2001, 2003, 2005; Bernards 2002; Boerjan et al. 2003; Davin and Lewis 2003; Dixon and Reddy 2003; Ralph et al. 2004a, b, Bunzel et al. 2005; Chiang 2006; Bourgaud et al. 2006; Suzuki and Umezawa 2007; Ferrer et al. 2008). As illustrated in Fig. 1, many parallel routes can be envisaged for this pathway. The pathway towards sinapyl alcohol via ferulic acid, sinapic acid, and sinapaldehyde had long been proposed for angiosperm syringyl lignin biosynthesis (Sarkanen 1971;

Higuchi 1985, 1997). This was based on tracer experiments with isotope-labelled phenylpropanoid monomers and associated enzymatic experiments (Sarkanen 1971; Higuchi 1985, 1997). For example, administration of ^{14}C-labelled *p*-hydroxycinnamic acids such as ferulic, 5-hydroxyferulic, and sinapic acids to various plant species, including wheat (*Triticum vulgare* var. Thatcher) resulted in the formation of ^{14}C-labelled syringyl lignin (Brown and Neish 1959; Higuchi and Brown 1963).

The new route for sinapyl alcohol biosynthesis

The first revision of the cinnamate/monolignol pathway resulted from the discovery of a new *O*-methyltransferase (OMT), caffeoyl CoA *O*-methyltransferase (CCoAOMT). This OMT methylates caffeoyl CoA to form feruloyl CoA, and 5-hydroxyferuloyl CoA to form sinapoyl CoA. At first, this new OMT was thought to play a role in defense. Later, the involvement of CCoAOMT in lignin biosynthesis was demonstrated (Ye et al. 1994, 2001).

Around that time, lignins in transgenic *Nicotiana tabacum* (tobacco) plants with altered expressions of phenylalanine ammonia-lyase (PAL) and cinnamate 4-hydroxylase (C4H) were analyzed. The results of those studies suggested that biosynthesis of monolignols from phenylalanine might occur by more than one route (Howles et al. 1996; Sewalt et al. 1997a). In addition, characteristics of lignin synthesis in a number of transgenic plants with downregulated expressions of caffeic acid *O*-methyltransferase (CAOMT or COMT) and/or CCoAOMT were reported. The downregulation of CAOMT in *N. tabacum* cv. Havana (Dwivedi et al. 1994), *Populus tremula* × *Populus alba* (Van Doorsselaere et al. 1995; Marita et al. 2001), *Populus tremuloides* (Tsai et al. 1998), *N. tabacum* cv. Samsun NN (Atanassova et al. 1995), and *N. tabacum* cv. Xanthi (Zhong et al. 1998) caused significant decreases in the S/G ratio, no large change in lignin content (0–11% decrease), and the appearance of a 5-hydroxyguaiacyl unit. On the other hand, suppression of CCoAOMT in *N. tabacum* cv. Xanthi (Zhong et al. 1998) and *P. tremula* × *P. alba* (Zhong et al. 2000; Meyermans et al. 2000) resulted in decreased lignin content and lower levels of guaiacyl and syringyl lignins.

These results suggested that CCoAOMT was involved in the biosynthesis of guaiacyl and syringyl lignins at least in these plant species, while CAOMT catalyzed the second methylation leading to the syringyl unit, although there were reports of different results that in CAOMT-silenced transgenic *N. tabacum* cv. Xanti and *P. tremula* × *P. alba*, substantial lignin reduction occurred (17–35% decrease) (Ni et al. 1994; Sewalt et al. 1997b; Jouanin et al. 2000). At this point, the role-sharing of CCoAOMT and CAOMT in the lignin biosynthetic pathway remained unclear.

The roles of CCoAOMT and CAOMT were clarified by the discovery of a novel pathway for syringyl lignin biosynthesis in angiosperm trees. In this pathway, syringyl lignin is synthesized via coniferaldehyde, 5-hydroxyconiferaldehyde, sinapaldehyde and sinapyl alcohol (Osakabe et al. 1999; Li et al. 2000, 2001; Chiang 2006). Similar pathways were also proposed for the herbaceous model plant *Arabidopsis thaliana* (Humphreys et al. 1999).

The discovery of the novel pathway via 5-hydroxyconiferaldehyde was based on kinetic analyses of recombinant and plant CAOMTs and ferulic acid 5-hydroxylases (F5Hs) of various angiosperm trees including *Liquidambar styraciflua* (sweetgum) and *P. tremuloides* (aspen) (Osakabe et al. 1999; Li et al. 2000). Individually incubating caffeic acid, 5-hydroxyferulic acid, and 5-hydroxyconiferaldehyde with CAOMT in the presence of *S*-adenosyl-L-methionine gave rise to the corresponding methylation products; ferulic acid, sinapic acid, and sinapaldehyde, respectively. When incubated individually with F5H, ferulic acid and coniferaldehyde were efficiently hydroxylated yielding 5-hydroxyferulic acid and 5-hydroxyconiferaldehyde, respectively. However, 5-hydroxyconiferaldehyde inhibited the CAOMT-catalyzed methylation of caffeic acid and 5-hydroxyferulic acid, while coniferaldehyde inhibited the F5H-catalyzed 5-hydroxylation of ferulic acid. This indicated that 5-hydroxyconiferaldehyde is the preferred substrate of CAOMT, and that coniferaldehyde is the preferred substrate of F5H (Osakabe et al. 1999; Li et al. 2000). Therefore CAOMT was renamed 5-hydroxyconiferaldehyde OMT (CAldOMT), and F5H was renamed coniferaldehyde 5-hydroxylase (CAld5H) (Osakabe et al. 1999; Li et al. 2000, 2001).

On the other hand, a different lignin biosynthetic pathway was reported for *Medicago sativa* (alfalfa). Significant lignin reduction (24–26% decrease) was observed in CAldOMT-silenced transgenic *M. sativa* (Guo et al. 2001; Chen et al. 2006). In contrast, downregulation of CCoAOMT led to reduced lignin levels (14–21% decrease) and a reduction in guaiacyl units without decreasing the amount of syringyl units (Guo et al. 2001; Chen et al. 2006). On the basis of these results, Guo et al. proposed a new biosynthetic pathway for syringyl lignin formation that was independent of guaiacyl lignin biosynthesis. In this new pathway, syringyl lignin is formed via caffealdehyde, coniferaldehyde, 5-hydroxyconiferaldehyde, sinapaldehyde and sinapyl alcohol. The new pathway differs from those proposed previously for angiosperm trees (Osakabe et al. 1999; Li et al. 2000) and *A. thaliana* (Humphreys et al. 1999) in two ways: caffealdehyde is involved as an intermediate and CCoAOMT does not participate in syringyl lignin formation (Guo et al. 2001; Parvathi et al. 2001; Dixon et al. 2001). Later, Chen et al. proposed that 3-methylation of the 3,4-dihydroxyphenyl moiety can be catalyzed by either CAOMT or another OMT in *M. sativa* (Chen et al. 2006). By contrast, Marita et al. (2003) reported that downregulation of CCoAOMT in *M. sativa* resulted in the reduction of both guaiacyl and syringyl units and thereby proposed a syringyl lignin formation pathway where CCoAOMT operated in addition to guaiacyl lignin formation. The biosynthetic pathway in *M. sativa* proposed by Marita et al. (2003) is essentially the same as those proposed for angiosperm trees (Osakabe et al. 1999; Li et al. 2000) and *A. thaliana* (Humphreys et al. 1999).

As well as methylation of 5-hydroxyconiferaldehyde, CAldOMTs can also efficiently transform 5-hydroxyconiferyl alcohol into the corresponding methylated alcohol, sinapyl alcohol (Humphreys et al. 1999; Parvathi et al. 2001; Zubieta et al. 2002; Nakatsubo et al. 2007a; Nakatsubo et al. 2008). In addition, using a radio-tracer method, Matsui et al. (2000) suggested the conversion of coniferyl alcohol to sinapyl alcohol in dicotyledonous angiosperms. However, identification of a sinapyl alcohol dehydrogenase (SAD) in *P. tremuloides*, a cinnamyl alcohol dehydrogenase (CAD) catalyzing the selective reduction of sinapaldehyde to sinapyl alcohol, reinforced the pathway from coniferaldehyde to sinapyl alcohol via 5-hydroxyconiferaldehyde and sinapaldehyde at least in tree species (Li et al. 2001; Chiang 2006). Although sinapaldehyde-specific CAD activity (i.e. true SAD) has not yet been found from other plant species, CAD with catalytic activity towards both coniferaldehyde and sinapaldehyde are known to exist, for example, in *A. thaliana* (Kim et al. 2004) and *Oryza sativa* (Zhang et al. 2006b).

Kinetic analyses of a recombinant *A. thaliana* CAldOMT encoded by At5g54160 indicated that 5-hydroxyconiferaldehyde inhibited the AtCAldOMT-catalyzed methylation of 5-hydroxyconiferyl alcohol with a K_i of 0.5 μM, and that the K_i value of the inhibition of 5-hydroxyconiferaldehyde methylation by 5-hydroxyconiferyl alcohol was 73.4 μM. Thus, the *O*-methylation of 5-hydroxyconiferyl alcohol by AtCAldOMT was significantly inhibited in the presence of 5-hydroxyconiferaldehyde. That is, when both substrates were present, AtCAldOMT preferentially catalyzed the *O*-methylation of 5-hydroxyconiferaldehyde (Nakatsubo et al. 2008). Similar results were obtained for *Carthamus tinctorius* (safflower) CAldOMT-catalyzed methylation of the two substrates (Nakatsubo et al. 2007a). These results further support the physiological role of the 5-hydroxyconiferaldehyde pathway for syringyl lignin biosynthesis, provided that 5-hydroxyconiferaldehyde and 5-hydroxyconiferyl alcohol exist together at the reaction site. The 5-hydroxyconiferaldehyde pathway for syringyl lignin biosynthesis is now considered to be a major in vivo pathway (Osakabe et al. 1999; Humphreys et al. 1999; Li et al. 2000, 2001; Ralph et al. 2001; Marita et al. 2003; Boerjan et al. 2003; Umezawa 2005; Chiang 2006).

It is often thought that syringyl lignin is found only in angiosperms. However, it has also been detected in lycophytes. Recently, a distinct CAld5H (F5H) was reported in the lycophyte *Selaginella moellendorffii*, which is a basal vascular plant with syringyl units in its lignin (Weng et al. 2008b). Phylogenetic analysis suggested that SmCAld5H evolved independently of angiosperm CAld5Hs (Weng et al. 2008b).

The new route for 3-hydroxylation of the *p*-hydroxyphenyl moiety

The 3-hydroxylation had been an enigmatic step in the cinnamate/monolignol pathway (Ehlting et al. 2006). Soon after the establishment of the pathway

from coniferaldehyde to sinapyl alcohol, a new route for 3-hydroxylation of the *p*-hydroxyphenyl moiety was proposed (Schoch et al. 2001; Franke et al. 2002b). The *A. thaliana* CYP98A3 was found to hydroxylate *p*-coumaric acid esters, especially the coumaroyl esters of shikimic and quinic acids (Schoch et al. 2001; Franke et al. 2002a). Similar substrate specificity was also reported for *Ocimum basilicum* CYP98A13 (Gang et al. 2002). In the wild-type, AtCYP98A3 was expressed in inflorescence stems and wounded tissues (Schoch et al. 2001) and lignified interfascicular fibers and xylem vessels in the stem (Nair et al. 2002). A mutant defective in the *AtCYP98A3* gene deposited smaller amounts of lignin compared with the wild-type, and the mutant lignin was formed primarily from *p*-coumaryl alcohol (Franke et al. 2002b). Similarly, down-regulation of the homologous genes in *M. sativa* cv. Regan SY (Ralph et al. 2006) and *Populus grandidentata* × *alba* (hybrid poplar) (Coleman et al. 2008) increased the proportion of *p*-hydroxyphenyl units relative to the normally dominant guaiacyl and syringyl units.

Later, a cDNA encoding hydroxycinnamoyl CoA: shikimate/quinate hydroxycinnamoyl transferase (HCT) was isolated from *N. tabacum* (Hoffmann et al. 2003). Downregulation of the *HCT* gene in *Nicotiana benthamiana* resulted in lower lignin content but a higher proportion of *p*-hydroxyphenyl lignin. In addition, an *A. thaliana* mutant in which the corresponding *A. thaliana* HCT was downregulated showed lower syringyl lignin content (Hoffmann et al. 2004), indicating that HCT was involved in lignification. The new pathway consisted of the ester exchange catalyzed by HCT and the subsequent 3-hydroxylation of the formed *p*-coumaroyl ester of shikimic acid or quinic acid by CYP98A3. This gives rise to the corresponding caffeoyl ester, followed by its conversion to caffeoyl CoA by HCT (Fig. 1) (Schoch et al. 2001; Franke et al. 2002b; Hoffmann et al. 2004). Thus, CYP98A3 was designated as *p*-coumaroyl shikimate/quinate 3-hydroxylase (C3′H) (Schoch et al. 2001). This enzyme is also often known as *p*-coumarate 3-hydroxylase (C3H) (Franke et al. 2002a, b).

Recently, the *Medicago truncatula HCT* gene was used to silence the endogenous *HCT* gene in *M. sativa* cv. Regen SY plants, and the *Pinus radiata HCT* gene was silenced in tracheary element-forming *P. radiata* callus culture. In both cases, silencing of these *HCT* genes resulted in an increased proportion of *p*-hydroxyphenyl lignin units but lower lignin contents (Chen et al. 2006; Wagner et al. 2007). These results suggested that the new pathway is conserved among diverse plant groups.

The initially proposed pathway included both shikimic and quinic acid esters as possible intermediates (Schoch et al. 2001; Franke et al. 2002b; Hoffmann et al. 2004). Later, cDNAs encoding hydroxycinnamoyl CoA:quinate hydroxycinnamoyl transferase (HQT) were isolated from *N. tabacum* and *Lycopersicon esculentum* (tomato) (Niggeweg et al. 2004). Transgenic *L. esculentum* plants were generated in which the *LeHQT* gene was silenced or overexpressed. Both types of transformants had similar lignin contents to that of the wild-type. The amount of chlorogenic acid (caffeoyl quinate) was decreased in the silenced plants, whereas it was increased in overexpressing plants (Niggeweg et al. 2004). Together, those results suggested that HQT was involved in chlorogenic acid formation, but did not supplement the activity of HCT in lignin biosynthesis (Niggeweg et al. 2004). Niggeweg et al. (2004) suggested that the *p*-coumaroyl ester of shikimic acid was the intermediate for lignin formation in the figure in their report, although it was not mentioned in the text.

Additional routes and enzymes in the cinnamate/monolignol pathway

Figure 1 shows revised routes for lignin biosynthesis in the cinnamate/monolignol pathway. These revisions were based on the cumulative results from a number of plant species including *A. thaliana*, *Populus* spp., and *N. tabacum*. It is plausible that these same routes operate in other plant species, but routes of lignin biosynthesis should still be validated in other plants. It is also possible that physiologically important routes in the pathway and genes for lignin synthesis in secondary xylem formation might differ from those for biosynthesis of other phenylpropanoid compounds, such as lignans, norlignans, and neolignans. As well, biosynthetic routes and genes may differ in lignin formation after fungal attack or physical wounding.

For example, Kawasaki et al. (2006) reported that *O. sativa CCR1* is involved in lignin synthesis

associated with the defense response but not in developmental lignin synthesis. In addition, metabolic analyses of the cinnamate/monolignol and lignan pathways were carried out to characterize biosynthetic processes in maturing seeds of *C. tinctorius*. These analyses, which included a stable-isotope-dilution method combined with the administration of stable-isotope-labelled precursors, strongly suggested that ferulic acid was the intermediate in lignan biosynthesis in *C. tinctorius* (Sakakibara et al. 2007).

Further research showed that AtCAldOMT can compensate for the function of AtCCoAOMT, which methylates the 3-hydroxyl group of the 3,4-dihydroxyphenyl moiety. This suggests the possible involvement of another shunt for sinapyl alcohol formation (Do et al. 2007; Nakatsubo et al. 2008). The At5g54160 gene had been annotated as a *CAOMT* (*CAldOMT*) gene based on its high sequence homology to the *P. tremuloides CAOMT* (*CAldOMT*) (Zhang et al. 1997). Later, it was reported that a recombinant protein of the gene methylated flavonoids (Muzac et al. 2000). Recently, the function of this gene was re-evaluated. Its recombinant enzyme catalyzed the *O*-methylation of phenylpropanoid monomers and flavonoid substrates with high efficiencies. The specificity constants (k_{cat}/K_m) for 5-hydroxyconiferaldehyde and quercetin were both 0.11 μM^{-1} min^{-1}. Lignins of At5g54160-knockout *A. thaliana* mutants lacked syringyl units. Taken together, these results strongly suggested that the gene was involved in both syringyl lignin and flavonoid biosynthesis, which was consistent with the results of gene co-expression network analysis (Tohge et al. 2007). Interestingly, silencing of the At5g54160 gene also resulted in significant accumulation of caffeyl alcohol, strongly suggesting that AtCAldOMT encoded by At5g54160 can methylate both 5-hydroxyconiferaldehyde and 3,4-dihydroxyphenyl compound(s) in syringyl lignin synthesis (Nakatsubo et al. 2008). These findings agreed with a recent report that an At4g34050 (the gene encoding *A. thaliana CCoAOMT*)-knockout mutant produced both guaiacyl and syringyl lignins, but had slightly lower total lignin content, while the At5g54160 (*AtCAldOMT*)-knockout mutant showed drastically decreased syringyl lignin content (Do et al. 2007). In addition, the At4g34050 and At5g54160 double-knockout mutant had very low lignin content, and its development was arrested at the plantlet stage. These results indicated that other members of *AtCCoAOMT* and *AtCAldOMT* gene families can not compensate for the functions of *AtCCoAOMT* (At4g34050) and *AtCAldOMT* (At5g54160). Importantly, the AtCCoAOMT-catalyzed methylation of 3,4-dihydroxyphenyl units in the wild type was replaced by AtCAldOMT in the *AtCCoAOMT*-knockout mutant (Do et al. 2007). Together, these results indicated that AtCAldOMT can catalyze methylation of 3,4-dihydroxyphenyl in vivo. A similar methylation of 3-hydroxyl group of the 3,4-dihydroxyphenyl moiety, that is, caffealdehyde methylation to coniferaldehyde catalyzed by CAldOMT in *M. sativa* was proposed (Guo et al. 2001; Parvathi et al. 2001), and should be tested in other plant species.

There may be additional enzymes that catalyze reactions in the cinnamate/monolignol pathway. For example, an OMT potentially involved in lignification has been identified in *Pinus taeda* (Li et al. 1997). This OMT methylated caffeic and 5-hydroxyferulic acids and caffeoyl and 5-hydroxyferuloyl CoA esters with similar specific activities; therefore, it was named hydroxycinnamic acids/hydroxycinnamoyl CoA esters OMT (AEOMT). The deduced protein sequence of AEOMT is partially similar to, but clearly distinguishable from, that of CAldOMTs. It does not exhibit any significant similarity to CCoAOMT protein sequences (Li et al. 1997). A putative promoter fragment of *AEOMT* was isolated from the *P. radiata* gene (Moyle et al. 2002). Histochemical analysis in transgenic *N. tabacum* plants revealed that the *AEOMT* promoter induced *GUS* expression in cell types associated with lignification, such as developing vessels, phloem and wood fibers, xylem parenchyma and non-lignifying phloem parenchyma, suggesting the possible role of the OMT in biosynthesis of phenylpropanoid compounds including monolignols (Moyle et al. 2002).

In addition, an OMT with higher preference for caffeic acid than for 5-hydroxyferulic acid was isolated from *M. sativa* cv. Apollo (alfalfa) (Inoue et al. 2000). This enzyme (COMT II) differed from CAOMT (CAldOMT) in its very low K_m for caffeic acid and its native molecular weight. MsCOMT II may be a true caffeic acid OMT (Inoue et al. 2000).

A cDNA encoding another OMT that is possibly involved in monolignol biosynthesis was cloned from

C. tinctorius. Phylogenetic analysis showed that the novel OMT did not belong to the typical CAldOMT cluster. The recombinant protein of the OMT catalyzed 3- (or 5-) *O*-methylation of hydroxycinnamaldehydes and hydroxycinnamyl alcohols, while it showed only weak or moderate activity towards hydroxycinnamates and hydroxycinnamoyl CoA esters (Nakatsubo et al. 2007b).

Similarly, an additional type of CAD was reported (Goffner et al. 1992, 1998; Damiani et al. 2005). The CAD, named CAD1, was first purified from *Eucalyptus gunnii* xylem. It reduced coniferaldehyde to coniferyl alcohol, but showed no activity towards sinapaldehyde (Goffner et al. 1992). Interestingly, a cDNA encoding EgCAD1 showed highest similarity to *CCR*s, and not the conventional *CAD*s (Goffner et al. 1998). The corresponding *CAD1* cDNAs were also isolated from *N. tabacum*, and the recombinant NtCAD1 showed activity towards coniferaldehyde and coniferyl alcohol, but not sinapaldehyde (Damiani et al. 2005).

As mentioned above, we can envision the outline of the cinnamate/monolignol pathway, especially for lignin biosynthesis. Our knowledge of the biosynthetic routes is largely based on the results of studies on enzyme kinetics and transgenic plants. The completion of entire genome sequences of some plant species, including *A. thaliana*, has enabled us to take advantage of the cDNAs already cloned and characterized in other species. These studies have provided lists of genes for which the functions have been annotated (Dixon and Reddy 2003; Goujon et al. 2003; Raes et al. 2003). For example, Raes et al. (2003) listed up to 61 gene family members that possibly encode *A. thaliana* cinnamate/monolignol pathway enzymes. Functions of a number of the annotated *A. thaliana* genes in the pathway have been characterized experimentally using their recombinant proteins, for instance, for C3H (Schoch et al. 2001; Franke et al. 2002a), CAldOMT (Nakatsubo et al. 2008), CAld5H (Humphreys et al. 1999), 4CL (Ehlting et al. 1999; Schneider et al. 2003; Hamberger and Hahlbrock 2004), CCR (Lauvergeat et al. 2001), and CAD (Kim et al. 2004). Kim et al. (2004) prepared recombinant proteins of nine *A. thaliana* CAD homologs, six of which were biochemically competent to reduce *p*-hydroxycinnamaldehyde substrates. Of those six, two had the highest activity and homology relative to CADs from other plant species (Kim et al. 2004). However, functions of many of the annotated genes in the pathway are yet to be determined experimentally, including biochemical characterization of their recombinant proteins. Hence, the identification of the *bona fide* functions of annotated genes has become one of the key subjects in plant biosciences. Systems-biological strategies are being used to unravel the complex and comprehensive control mechanisms that underlie metabolic processes of plants.

MYB transcription factors in the control of the cinnamate/monolignol pathway and lignin biosynthesis

Transcription factors (Kranz et al. 1998; Rogers and Campbell 2004; Olsen et al. 2005; Zhong and Ye 2007) and microRNAs (Lu et al. 2005; Zhang et al. 2006a; Groover and Robischon 2006) play important roles in the comprehensive control of plant metabolism. Lu et al. (2005) reported microRNA-mediated control of cell wall formation, and there are many recent reports on metabolic control of cell wall formation by transcription factors (Rogers and Campbell 2004; Zhong and Ye 2007). The MYB family of transcription factors is one of the most abundant classes of transcription factors in plants. The largest subfamily is R2R3-MYB, which contains the two-repeat R2R3 DNA-binding domain (Stracke et al. 2001). A number of *R2R3-MYB* genes that regulate phenylpropanoid biosynthesis in plants were outlined by Rogers and Campbell (2004).

Tamagnone et al. (1998) demonstrated that overexpression of an *Antirrhinum majus R2R3-MYB* gene (*AmMYB308*) repressed lignin biosynthesis in transgenic *N. tabacum* plants. The expression of the *PAL* gene was not affected by *AmMYB308*-overexpression in tobacco plants, whereas the expressions of *C4H*, *4CL,* and *CAD* genes were significantly reduced in plants overexpressing *AmMYB308* (Tamagnone et al. 1998). Similarly, 4CL gene expression was reduced in *N. tabacum* plants overexpressing *AmMYB330* (Tamagnone et al. 1998). Experiments with yeast indicated that AmMYB308 can act as a very weak transcriptional activator. Thus, it was hypothesized that AmMYB308 out-competes endogenous strong activators and thereby competitively inhibits gene activation (Tamagnone et al. 1998).

While *AmMYB308* was overexpressed in *N. tabacum* (Tamagnone et al. 1998), Jin et al. (2000) analyzed an *A. thaliana* mutant defective in the *A. thaliana* ortholog of *AmMYB308*, *AtMYB4*. The mutant had enhanced levels of sinapate esters in its leaves. In addition, AtMYB4 negatively regulated the *C4H* gene, but positively regulated the *CCoAOMT* gene (Jin et al. 2000). On the other hand, the transcript level of CCoAOMT in the T-DNA insertion mutant of *AtMYB32* (a gene with high similarity to *AtMYB4*) was unchanged, while the transcript level of CAOMT was increased (Preston et al. 2004). Recently, increased expression of *AtMYB4* was found in *A. thaliana* in response to cadmium exposure (van de Mortel et al. 2008).

Another *A. thaliana* R2R3-MYB, AtPAP1 (=AtMYB75), has a marked effect on activating flavonoid biosynthesis (Borevitz et al. 2000; Tohge et al. 2005). In the *A. thaliana* mutant *pap1-D*, the *AtPAP1* gene shows massive and widespread activation throughout plant development. This mutant was characterized by strongly increased concentrations of glycosylated anthocyanins and flavonols compared with the wild-type, and also by increased lignin content (Borevitz et al. 2000). Although both accumulation of *PAL* transcripts and PAL activity in *pap1-D* were increased compared with the wild-type (Borevitz et al. 2000), the genes involved in the monolignol biosynthetic pathway were not upregulated by AtPAP1, except *4CL* (Tohge et al. 2005). This result suggests that upregulation of PAL enhances lignin accumulation.

Patzlaff et al. (2003b) characterized a member of the R2R3-MYB family, PtMYB4, from *P. taeda*. PtMYB4 may play a direct role in regulating lignin biosynthesis during lignification. This MYB was expressed in lignifying *P. taeda* cells. In transgenic *N. tabacum* plants overexpressing *PtMYB4*, the expression of *C3H*, *CCoAOMT*, *CAOMT*, *CCR*, and *CAD* was increased, while *PAL* expression was decreased. In addition, the transgenic *N. tabacum* plants accumulated greater quantities of lignin and showed ectopic lignification. Together, these results strongly suggested that *PtMYB4* enables lignification (Patzlaff et al. 2003b).

Overexpression of *PtMYB4* in *A. thaliana* caused ectopic lignin deposition, and the *PtMYB4* overexpressor shared some phenotypic traits, including ectopic lignification, with the *A. thaliana de-etiolated 3* (*det3*) mutant (Newman et al. 2004). Expression analyses of *A. thaliana R2R3-MYB* genes that showed high similarity to *PtMYB4* suggested that *AtMYB61* was misexpressed in the *det3* mutant. Overexpression of *AtMYB61* produced a phenocopy of the *det3* mutant, including the ectopic accumulation of lignin. Taken together, these results suggested that *AtMYB61* has a role in regulation of lignin biosynthesis in *A. thaliana* (Newman et al. 2004).

Several other *R2R3-MYB*s have been characterized from conifers. *PtMYB1* was suggested to regulate lignin biosynthesis (Patzlaff et al. 2003a). Later, the roles of *PtMYB1* and *PtMYB8* were further studied using transformants of another gymnosperm *Picea glauca* (spruce) (Bomal et al. 2008). Overexpression of *PtMYB1* and *PtMYB8* in *P. glauca* caused ectopic secondary wall deposition and increased lignin content. In addition, overexpression of a number of genes including *PAL*, *4CL*, and *CAOMT* was observed in the *PtMYB1*- and *PtMYB8*-overexpressors, suggesting that *PtMYB1* and *PtMYB8* may be involved in secondary wall deposition (Bomal et al. 2008). *P. glauca MYB*s, *PgMYB1*, *PgMYB2*, *PgMYB4* and *PgMYB8*, which are the closest homologs to *PtMYB1*, *PtMYB2*, *PtMYB4* and *PtMYB8*, respectively, were expressed specifically in xylem tissues (Bedon et al. 2007).

In the hybrid aspen *P. tremula* × *P. tremuloides*, an *R2R3-MYB* designated as *PttMYB21a* was expressed strongly in xylem tissues. Transgenic aspen plants expressing the *MYB* gene in an antisense orientation showed higher transcript levels of CCoAOMT in the phloem, suggesting that *PttMYB21a* might work as a repressor (Karpinska et al. 2004).

Goicoechea et al. (2005) and Legay et al. (2007) reported two R2R3-MYB family members, EgMYB1 and EgMYB2, from *E. gunnii*. EgMYB2 was expressed preferentially in the differentiating secondary xylem in stems and roots. The recombinant EgMYB2 protein specifically bound to the *cis*-regulatory regions of the promoters of *EgCCR* and *EgCAD2* (Goicoechea et al. 2005). Overexpression of *EgMYB2* in *N. tabacum* plants caused ectopic lignification and drastic upregulation of genes encoding enzymes committed to monolignol synthesis; *C3H*, *CCoAOMT*, *F5H*, *CAOMT*, *HCT*, *CCR*, *CAD*, and *4CL*. Taken together, the results strongly suggested that *EgMYB2* is involved in transcriptional regulation of lignin biosynthesis as a transcriptional activator (Goicoechea et al. 2005). On the other hand, *EgMYB1* may be a negative

regulator of lignin biosynthesis (Legay et al. 2007). *EgMYB1* was preferentially expressed in differentiating xylem tissues in stems and roots of *Eucalyptus*. EgMYB1 bound to *cis*-regulatory elements in the promoters of *EgCCR* and *EgCAD2* and repressed the transcription of these genes (Legay et al. 2007).

An *R2R3-MYB* family member from *Vitis vinifera* cv. Cabernet Sauvignon (grape), *VvMYB5a*, was expressed in skin, flesh, and seeds, mainly during the early stages of berry development (Deluc et al. 2006). Overexpression of the *MYB* gene in *N. tabacum* resulted in decreased expression of *CCoAOMT* in stamens (Deluc et al. 2006).

Fornalé et al. (2006) characterized two R2R3-MYB transcription factors ZmMYB31 and ZmMYB42 from *Zea mays* (maize). Expression of *CAOMT* genes was downregulated in *A. thaliana* plants overexpressing *ZmMYB31* and *ZmMYB42* genes. In addition, overexpression of the *MYB* genes also affected expression of other genes in the cinnamate/monolignol pathway, and resulted in decreased lignin content in transgenic plants (Fornalé et al. 2006).

LIM transcription factors in the control of the cinnamate/monolignol pathway and lignin biosynthesis

LIM proteins derive their name from the initials of the homeodomain proteins Lin11, Isl-1 and Mec-3 (Bach 2000). A member of the LIM proteins, NtLIM1, was isolated from *N. tabacum* on the basis of its capacity to bind to an AC element, which is an important *cis*-acting element for gene expression in lignification (Kawaoka et al. 2000; Kawaoka and Ebinuma 2001). Transgenic *N. tabacum* plants with antisense *NtLIM1* had low transcript levels of some lignin biosynthetic pathway genes, such as *PAL*, *4CL*, and *CAD*. Furthermore, these transformants showed a 27% reduction in lignin content (Kawaoka et al. 2000).

A homologous gene, *EcLIM1,* was isolated from *Eucalyptus camaldulensis* (Kawaoka et al. 2006). Antisense *NtLIM1* was introduced into *E. camaldulensis*, and transgenic *Eucalyptus* plants showed decreased expression levels of *EcLIM1* as well as several lignin biosynthesis genes, including *PAL*, *C4H*, and *4CL*, and a 29% reduction in lignin content. These results show that *EcLIM1* is one of the key transcription factors involved in lignin biosynthesis in *E. camaldulensis* (Kawaoka et al. 2006).

Transcriptional network regulating secondary wall biosynthesis including lignification

Recently, transcriptional profiles were determined for genes that are differentially expressed during secondary wall formation. These profiles led to the discovery of a hierarchy of transcription factors involved in regulating secondary wall biosynthesis, including lignification (Fig. 2) (Zhong and Ye 2007; Demura and Fukuda 2007; Yang et al. 2007; Zhong et al. 2008, 2009). Functional redundancy of transcription factors often complicates characterization of their functions. This can be overcome by using chimeric repressor silencing technology (Hiratsu et al. 2004).

Mitsuda et al. (2005) used the silencing technology to characterize two NAC transcription factors from *A. thaliana*, AtNST1 (NAC SECONDARY WALL THICKENING PROMOTING FACTOR 1) and NST2. The silencing results, and results from other techniques including phenotyping of T-DNA double knockout mutants, indicated that AtNST1 and AtNST2 are redundant in regulating secondary wall thickening in anther endothecium cell walls. Overexpression of *AtNST1* induced ectopic lignified secondary wall thickening in various tissues of *A. thaliana*, including stems. Overexpressors also showed upregulated expression of several genes involved in secondary wall biosynthesis, including *CCR* (Mitsuda et al. 2005). Subsequently, it was shown that AtMYB26 regulates expressions of *AtNST1* and *AtNST2* (Yang et al. 2007).

On the other hand, Kubo et al. (2005) found that two *A. thaliana* NAC transcription factors, AtVND6 (VASCULAR-RELATED NAC-DOMAIN 6) and AtVND7 induced transdifferentiation of various types of cells into metaxylem- and protoxylem-like vessel elements, respectively, in *A. thaliana* and *P. tremula* × *P. tremuloides* (hybrid aspen). These results suggest that AtVND6 and AtVND7 are transcriptional switches for plant metaxylem and

protoxylem vessel formation, respectively (Kubo et al. 2005).

Zhong et al. (2006) showed that expression of a NAC transcription factor gene of *A. thaliana*, *AtSND1* (*secondary wall-associated NAC domain protein 1*), is associated with secondary wall thickening in *A. thaliana* fibers. Dominant repression of AtSND1 in *A. thaliana* led to a severe decrease in the secondary wall thickness of fibers. Overexpression of AtSND1 in *A. thaliana* resulted in activation of genes including *CCoAOMT* and *4CL*, which are involved in lignin biosynthesis, leading to ectopic deposition of secondary walls in cells that are normally nonsclerenchymatous. As well, AtSND1 overexpression in *A. thaliana* resulted in upregulation of a number of transcription factors, such as NACs (AtSND2, AtSND3), MYBs (AtMYB20, AtMYB46, AtMYB85, AtMYB103), and a homeodomain transcription factor (AtKNAT7). Zhong et al. concluded that AtSND1 is a transcriptional activator involved in secondary wall thickening in fibers (Zhong et al. 2006, 2007a).

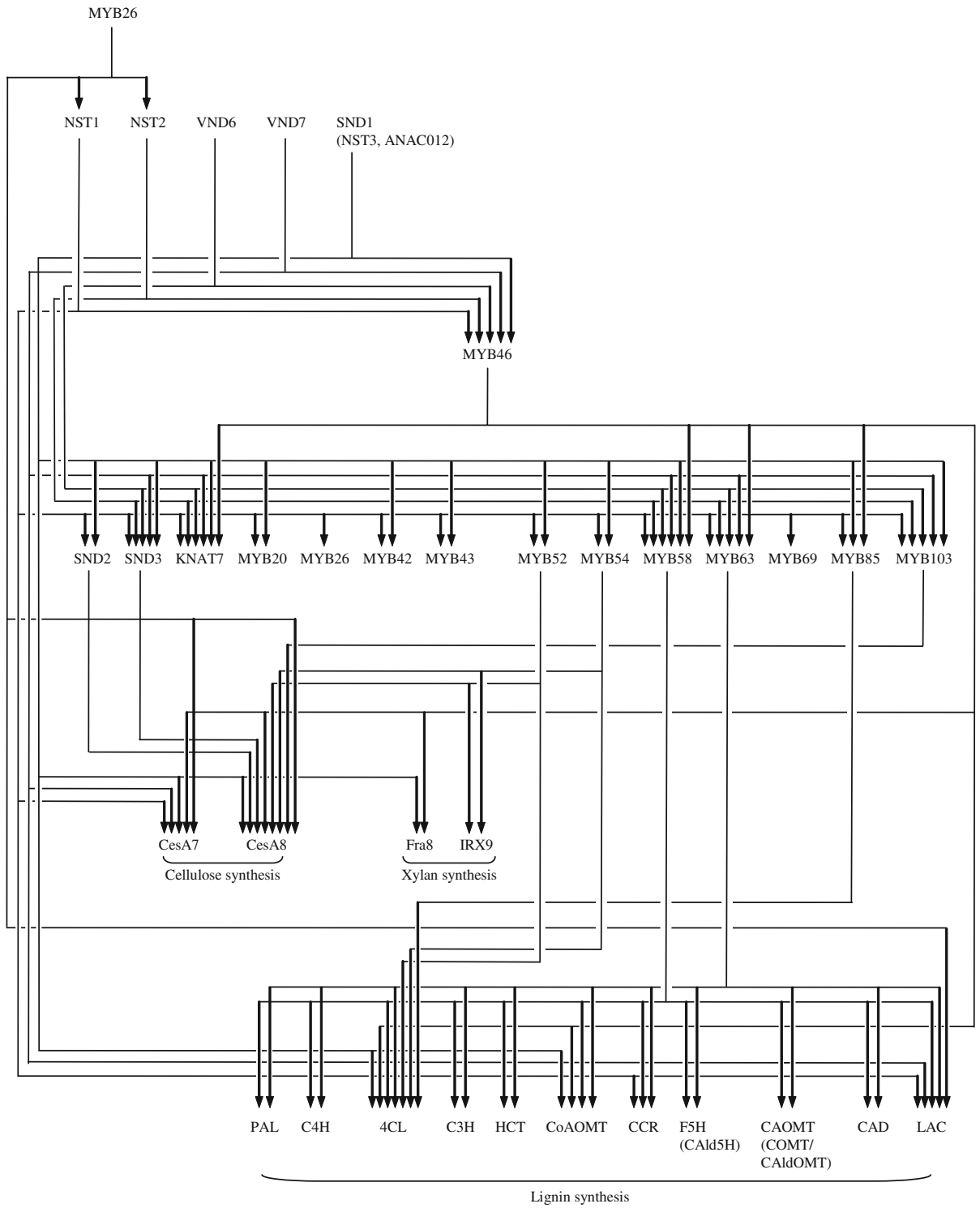
On the other hand, overexpression of AtSND1 inhibited normal secondary wall thickening in *A. thaliana* fibers (Zhong et al. 2006). Ko et al. (2007) reported a similar inhibition by AtSND1 (also known as AtANAC012), and concluded that AtANAC012 may act as a negative regulator of secondary wall thickening in *A. thaliana* xylary fibers (Ko et al. 2007), in contrast to the above-mentioned conclusion of Zhong et al. (2006).

As well as its role in regulating secondary wall thickening in anther endothecium cell walls, AtNST1 has a role in regulating formation of *A. thaliana* fibers. AtNST1 and AtSND1 (also known as AtNST3 and AtANAC012) redundantly regulate secondary wall thickening in fibers (Mitsuda et al. 2007; Zhong et al. 2007b).

Secondary wall biosynthesis research has subsequently focused on identifying factors downstream in the hierarchy of transcriptional regulation (Fig. 2) (Zhong and Ye 2007; Demura and Fukuda 2007; Zhong et al. 2008; Zhou et al. 2009). First, AtMYB46 was found to be a direct target of AtSND1. In addition, AtSND1 and its close homologs AtNST1, AtNST2, AtVND6, and AtVND7, were able to activate the *AtMYB46* promoter. Dominant repression of AtMYB46 caused a drastic reduction in the secondary wall thickening of both fibers and vessels in *A. thaliana*, while overexpression of AtMYB46 activated the biosynthetic pathways of cellulose, xylan, and lignin. Thus, the expression of cellulose synthase genes *CesA7* and *CesA8*, the xylan biosynthetic gene *FRA8*, and lignin biosynthetic genes *4CL1* and *CCoAOMT* were drastically induced (Fig. 2). Concomitantly, overexpression of AtMYB46 in *A. thaliana* led to ectopic deposition of secondary walls in cells that are normally nonsclerenchymatous (Zhong et al. 2007a).

Fig. 2 Hierarchy of transcriptional activation in biosynthesis of lignin, cellulose and xylan in *Arabidopsis thaliana*. This regulation diagram is a summary of overexpression and direct promoter activation experiments of the transcription factors in addition to their chimeric repressor silencing, double RNAi inhibition, and double T-DNA knockout experiments (Mitsuda et al. 2005; Kubo et al. 2005; Zhong et al. 2006, 2007a, b, 2008; Mitsuda et al. 2007; Yang et al. 2007; Zhou et al. 2009). CesA7, Cellulose synthase catalytic subunit 7; CesA8, Cellulose synthase 8; Fra8, Fragile fiber 8 (a member of glycosyltransferase family 47); IRX9, putative family 43 glycosyl transferase; the abbreviations of enzymes involved in lignin synthesis are described in Fig. 1 legend

In addition to AtMYB46, three transcription factors, AtSND3, AtMYB103, and AtKNAT7, were found to be direct targets of AtSND1, AtNST1, AtNST2, AtVND6, and AtVND7, except that the activation of *AtSND3* by AtNST1 was indirect (Zhong et al. 2008). In *AtSND1* and *AtNST1* double RNAi plants, 11 transcription factors were downregulated as well as AtMYB46. These included two NACs (AtSND2 and AtSND3), eight MYBs (AtMYB103, AtMYB85, AtMYB52, AtMYB54, AtMYB69, AtMYB42, AtMYB43, and AtMYB20), and a homeodomain transcription factor (AtKNAT7) (Zhong et al. 2008). Dominant repression of AtSND2, AtSND3, AtMYB103, AtMYB85, AtMYB52, AtMYB54, AtMYB69, and AtKNAT7 caused a severe reduction in secondary wall thickening in both interfascicular fibers and xylary fibers in inflorescence stems, whereas secondary wall thickening in vessels was not significantly affected. Overexpression of AtSND2, AtSND3, and AtMYB103 resulted in increased secondary wall thickening in fiber cells. In addition, overexpression of AtMYB85 brought about ectopic deposition of lignin in epidermal and cortical cells in stems. AtSND2, AtSND3, and AtMYB103 induced the GUS reporter gene expression driven by the promoter of a cellulose synthase gene, *CesA8*, whereas AtMYB85 specifically induced GUS



expression driven by the promoter of the lignin biosynthetic gene, *4CL*. AtMYB52 and AtMYB54 induced GUS expression driven by promoters of *CesA8*, *4CL*, and a xylan biosynthetic gene, *IRX9*. These results indicated that AtSND2, AtSND3, and AtMYB103 are involved in cellulose synthesis, while

AtMYB85 specifically induce lignin biosynthesis. On the other hand, AtMYB52 and AtMYB54 are involved in the biosynthesis of the three major cell wall components (Fig. 2) (Zhong et al. 2008).

Thus, AtSND1/NST3/ANAC012 and AtNST1 function redundantly in activating secondary wall biosynthesis in fibers (Zhong et al. 2006, 2007b; Mitsuda et al. 2007). AtVND6 and AtVND7 regulate the differentiation of the metaxylem and protoxylem, respectively, in primary roots (Kubo et al. 2005), while AtNST1 and AtNST2 function redundantly in regulating secondary wall thickening in endothecium cells of the anther (Mitsuda et al. 2005).

Recently, two AtSND1-regulated *A. thaliana* MYB transcription factors, AtMYB58 and AtMYB63, were found to specifically activate lignin biosynthetic genes during secondary wall formation in *A. thaliana* (Zhou et al. 2009). Expression studies showed that AtMYB58 and AtMYB63 were specifically expressed in fibers and vessels undergoing secondary wall thickening. Simultaneous RNAi inhibition of both AtSND1 and AtNST1 led to drastic reduction in the expressions of *AtMYB58* and *AtMYB63*. *AtMYB58* expression was significantly upregulated by overexpression of AtSND1 and AtNST1. On the other hand, AtMYB63 expression was induced by *AtNST1* overexpression but not by *AtSND1*. Dominant repression of *AtMYB58* and *AtMYB63* reduced secondary wall thickening of fibers and vessels. As well, the amount of guaiacyl lignin and syringyl lignin was significantly reduced in plants with dominant repression and simultaneous RNAi inhibition of *AtMYB58* and *AtMYB63*. Overexpression of AtMYB58 and AtMYB63 specifically activated lignin biosynthetic genes (*PAL1*, *C4H*, *4CL*, *HCT*, *C3H*, *CCoAOMT*, *CCR*, *CAOMT*, and *CAD*), alongside a concomitant ectopic deposition of lignin in cells that are normally unlignified. However, there was no activation of cellulose synthase genes *CesA4*, *CesA7*, and *CesA8*, or xylan biosynthetic genes *IRX8* and *IRX9*. In addition, AtMYB58 directly activated the expression of lignin biosynthetic genes. These results indicated that AtMYB58 and AtMYB63 are specific transcriptional activators of lignin biosynthesis in the transcription network that regulates secondary wall biosynthesis (Fig. 2) (Zhou et al. 2009).

Summary

The cinnamate/monolignol pathway has been studied intensively, especially in relation to lignification, and routes within the pathway have been revised several times over the last decade. In the post-genomic era, lists of genes for which functions have been annotated are available for some plant species, including *A. thaliana*. The identification of the *bona fide* functions of annotated genes of the cinnamate/monolignol pathway is being studied intensively in relation to the elucidation of comprehensive control mechanisms of plant metabolism, in which transcription factors play important roles. Recently, a number of transcription factors that function as the master switches for xylem formation have been discovered. At present, research is focused on the transcription factors downstream in the hierarchy of transcriptional controls regulating the biosynthesis of secondary wall components, including hydroxycinnamates and lignins.

References

Atanassova R, Favet N, Martz F, Chabbert B, Tollier M-T, Monties B, Fritig B, Legrand M (1995) Altered lignin composition in transgenic tobacco expressing O-methyltransferase sequences in sense and antisense orientation. Plant J 8:465–477

Bach I (2000) The LIM domain: regulation by association. Mech Develop 91:5–17

Bedon F, Grima-Pettenati J, Mackay J (2007) Conifer R2R3-MYB transcription factors: sequence analyses and gene expression in wood-forming tissues of white spruce (*Picea glauca*). BMC Plant Biol 7:17

Bernards MA (2002) Demystifying suberin. Can J Bot 80: 227–240

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Annu Rev Plant Biol 54:519–546

Bomal C, Bedon F, Caron S, Mansfield SD, Levasseur C, Cooke JEK, Blais S, Tremblay L, Morency M-J, Pavy N, Grima-Pettenati J, Séguin A, MacKay J (2008) Involvement of *Pinus taeda MYB1* and *MYB8* in phenylpropanoid metabolism and secondary cell wall biogenesis: a comparative *in planta* analysis. J Exp Bot 59:3925–3939

Borevitz JO, Xia Y, Blount J, Dixon RA, Lamb C (2000) Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 12:2383–2393

Bourgaud F, Hehn A, Larbat R, Doerper S, Gontier E, Kellner S, Matern U (2006) Biosynthesis of coumarins in plants: a

major pathway still to be unravelled for cytochrome P450 enzymes. Phytochem Rev 5:293–308

Brown SA, Neish AC (1959) Studies of lignin biosynthesis using isotopic carbon. VIII. Isolation of radioactive hydrogenolysis products of lignin. J Am Chem Soc 81:2419–2424

Bunzel M, Ralph J, Funk C, Steinhart H (2005) Structural elucidation of new ferulic acid-containing phenolic dimers and trimers isolated from maize bran. Tetrahedron Lett 46:5845–5850

Chen F, Reddy MSS, Temple S, Jackson L, Shadle G, Dixon RA (2006) Multi-site genetic modulation of monolignol biosynthesis suggests new routes for formation of syringyl lignin and wall-bound ferulic acid in alfalfa *(Medicago sativa* L.). Plant J 48:113–124

Chiang VL (2006) Monolignol biosynthesis and genetic engineering of lignin in trees, a review. Environ Chem Lett 4:143–146

Coleman HD, Park J-Y, Nair R, Chapple C, Mansfield SD (2008) RNAi-mediated suppression of *p*-coumaroyl-CoA 3'-hydroxylase in hybrid poplar impacts lignin deposition and soluble secondary metabolism. Proc Natl Acad Sci USA 105:4501–4506

Damiani I, Morreel K, Danoun S, Goeminne G, Yahiaoui N, Marque C, Kopka J, Messens E, Goffner D, Boerjan W, Boudet A-M, Rochange S (2005) Metabolite profiling reveals a role for atypical cinnamyl alcohol dehydrogenase CAD1 in the synthesis of coniferyl alcohol in tobacco xylem. Plant Mol Biol 59:753–769

Davin L, Lewis NG (2003) An historical perspective on lignan biosynthesis: monolignol, allylphenol and hydroxycinnamic acid coupling and downstream metabolism. Phytochem Rev 2:257–288

Deluc L, Barrieu F, Marchive C, Lauvergeat V, Decendit A, Richard T, Carde J-P, Mérillon J-M, Hamdi S (2006) Characterization of a grapevine R2R3-MYB transcription factor that regulates the phenylpropanoid pathway. Plant Physiol 140:499–511

Demura T, Fukuda H (2007) Transcriptional regulation in wood formation. Trends Plant Sci 12:64–70

Dixon RA, Reddy MSS (2003) Biosynthesis of monolignols. Genomic and reverse genetic approaches. Phytochem Rev 2:289–306

Dixon RA, Chen F, Guo D, Parvathi K (2001) The biosynthesis of monolignols: a "metabolic grid", or independent pathways to guaiacyl and syringyl units? Phytochemistry 57:1069–1084

Do C-T, Pollet B, Thévenin J, Sibout R, Denoue D, Barrière Y, Lapierre C, Jouanin L (2007) Both caffeoyl coenzyme A 3-*O*-methyltransferase 1 and caffeic acid *O*-methyltransferase 1 are involved in redundant functions for lignin, flavonoids and sinapoyl malate biosynthesis in *Arabidopsis*. Planta 226:1117–1129

Dwivedi UN, Campbell WH, Yu J, Datla RSS, Bugos RC, Chiang VL, Podila GK (1994) Modification of lignin biosynthesis in transgenic *Nicotiana* through expression of an antisense *O*-methyltransferase gene from *Populus*. Plant Mol Biol 26:61–71

Ehlting J, Büttner D, Wang Q, Douglas CJ, Somssich IE, Kombrink E (1999) Three 4-coumarate:coenzyme A ligases in *Arabidopsis thaliana* represent two evolutionarily divergent classes in angiosperms. Plant J 19:9–20

Ehlting J, Hamberger B, Million-Rousseau R, Werck-Reichhart D (2006) Cytochromes P450 in phenolic metabolism. Phytochem Rev 5:239–270

Ferrer J-L, Austin MB, Stewart C Jr, Noel JP (2008) Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. Plant Physiol Biochem 46:356–370

Fornalé S, Sonbol F-M, Maes T, Capellades M, Puigdomènech P, Rigau J, Caparrós-Ruiz D (2006) Down-regulation of the maize and arabidopsis thaliana caffeic acid *O*-methyltransferase genes by two new maize R2R3-MYB transcription factors. Plant Mol Biol 62:809–823

Franke R, Humphreys JM, Hemm MR, Denault JW, Ruegger MO, Cusumano JC, Chapple C (2002a) The Arabidopsis *REF8* gene encodes the 3-hydroxylase of phenylpropanoid metabolism. Plant J 30:33–45

Franke R, Hemm MR, Denault JW, Ruegger MO, Humphreys JM, Chapple C (2002b) Changes in secondary metabolism and deposition of an unusual lignin in the *ref8* mutant of Arabidopsis. Plant J 30:47–59

Gang DR, Beuerle T, Ullmann P, Werck-Reichhart D, Pichersky E (2002) Differential production of *meta* hydroxylated phenylpropanoids in sweet basil peltate glandular trichomes and leaves is controlled by the activities of specific acyltransferases and hydroxylases. Plant Physiol 130:1536–1544

Goffner D, Joffroy I, Grima-Pettenati J, Halpin C, Knight ME, Schuch W, Boudet AM (1992) Purification and characterization of isoforms of cinnamyl alcohol dehydrogenase from *Eucalyptus* xylem. Planta 188:48–53

Goffner D, Van Doorsselaere J, Yahiaoui N, Samaj J, Grima-Pettenati J, Boudet AM (1998) A novel aromatic alcohol dehydrogenase in higher plants: molecular cloning and expression. Plant Mol Biol 36:755–765

Goicoechea M, Lacombe E, Legay S, Mihaljevic S, Rech P, Jauneau A, Lapierre C, Pollet B, Verhaegen D, Chaubet-Gigot N, Grima-Pettenati J (2005) *Eg*MYB2, a new transcriptional activator from *Eucalyptus* xylem, regulates secondary cell wall formation and lignin biosynthesis. Plant J 43:553–567

Goujon T, Sibout R, Eudes A, MacKay J, Jouanin L (2003) Genes involved in the biosynthesis of lignin precursors in *Arabidopsis thaliana*. Plant Physiol Biochem 41:677–687

Groover A, Robischon M (2006) Developmental mechanisms regulating secondary growth in woody plants. Curr Opin Plant Biol 9:55–58

Guo D, Chen F, Inoue K, Blount JW, Dixon RA (2001) Downregulation of caffeic acid 3-*O*-methyltransferase and caffeoyl CoA 3-*O*-methyltransferase in transgenic alfalfa: impacts on lignin structure and implications for the biosynthesis of G and S lignin. Plant Cell 13:73–88

Hamberger B, Hahlbrock K (2004) The *4-coumarate:CoA ligase* gene family in *Arabidopsis thaliana* comprises one rare, sinapate-activating and three commonly occurring isoenzymes. Proc Natl Acad Sci USA 101:2209–2214

Higuchi T (1985) Biosynthesis of lignin. In: Higuchi T (ed) Biosynthesis and biodegradation of wood components. Academic, Orlando, pp 141–160

Higuchi T (1997) Biochemistry and molecular biology of wood. Springer, Berlin, pp 1–362

Higuchi T, Brown SA (1963) Studies of lignin biosynthesis using isotopic carbon. XII. The biosynthesis and metabolism of sinapic acid. Can J Biochem Physiol 41:613–620

Hiratsu K, Mitsuda N, Matsui K, Ohme-Takagi M (2004) Identification of the minimal repression domain of SUPERMAN shows that the DLELRL hexapeptide is both necessary and sufficient for repression of transcription in *Arabidopsis*. Biochem Biophys Res Commun 321:172–178

Hoffmann L, Maury S, Martz F, Geoffroy P, Legrand M (2003) Purification, cloning, and properties of an acyltransferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism. J Biol Chem 278:95–103

Hoffmann L, Besseau S, Geoffroy P, Rizenthaler C, Meyer D, Lapierre C, Pollet B, Legrand M (2004) Silencing of hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamoyltransferase affects phenylpropanoid biosynthesis. Plant Cell 16:1446–1465

Howles PA, Sewalt VJH, Paiva NL, Elkind Y, Bate NJ, Lamb C, Dixon RA (1996) Overexpression of L-phenylalanine ammonia-lyase in transgenic tobacco plants reveals control points for flux into phenylpropanoid biosynthesis. Plant Physiol 112:1617–1624

Humphreys JM, Hemm MR, Chapple C (1999) New routes for lignin biosynthesis defined by biochemical characterization of recombinant ferulate 5-hydroxylase, a multifunctional cytochrome P450-dependent monooxygenase. Proc Natl Acad Sci USA 96:10045–10050

Inoue K, Parvathi K, Dixon RA (2000) Substrate preferences of caffeic acid/5-hydroxyferulic acid 3/5-*O*-methyltransferases in developing stems of alfalfa (*Medicago sativa* L.). Arch Biochem Biophys 375:175–182

Ishii T (1997) Structure and functions of feruloylated polysaccharides. Plant Sci 127:111–127

Jin H, Cominelli E, Bailey P, Parr A, Mehrtens F, Jones J, Tonelli C, Weisshaar B, Martin C (2000) Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in *Arabidopsis*. EMBO J 19:6150–6161

Jouanin L, Goujon T, de Nadaï V, Martin M-T, Mila I, Vallet C, Pollet B, Yoshinaga A, Chabbert B, Petit-Conil M, Lapierre C (2000) Lignification in transgenic poplars with extremely reduced caffeic acid *O*-methyltransferase activity. Plant Physiol 123:1363–1373

Karpinska B, Karlsson M, Srivastava M, Stenberg A, Schrader J, Sterky F, Bhalerao R, Wingsle G (2004) MYB transcription factors are differentially expressed and regulated during secondary vascular tissue development in hybrid aspen. Plant Mol Biol 56:255–270

Kawaoka A, Ebinuma H (2001) Transcriptional control of lignin biosynthesis by tobacco LIM protein. Phytochemistry 57:1149–1157

Kawaoka A, Kaothien P, Yoshida K, Endo S, Yamada K, Ebinuma H (2000) Functional analysis of tobacco LIM protein Ntlim1 involved in lignin biosynthesis. Plant J 22:289–301

Kawaoka A, Nanto K, Ishii K, Ebinuma H (2006) Reduction of lignin content by suppression of expression of the LIM domain transcription factor in *Eucalyptus camaldulensis*. Silvae Genetica 55:269–277

Kawasaki T, Koita H, Nakatsubo T, Hasegawa K, Wakabayashi K, Takahashi H, Umemura K, Umezawa T, Shimamoto K (2006) Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is an effector of small GTPase Rac in defense signaling in rice. Proc Natl Acad Sci USA 103:230–235

Kim S-J, Kim M-R, Bedgar DL, Moinuddin SGA, Cardenas CL, Davin LB, Kang C, Lewis NG (2004) Functional reclassification of the putative cinnamyl alcohol dehydrogenase multigene family in *Arabidopsis*. Proc Natl Acad Sci USA 101:1455–1460

Ko J-H, Yang SH, Park AH, Lerouxel O, Han K-H (2007) ANAC012, a member of the plant-specific NAC transcription factor family, negatively regulates xylary fiber development in *Arabidopsis thaliana*. Plant J 50:1035–1048

Kranz HD, Denekamp M, Greco R, Jin H, Leyva A, Meissner RC, Petroni K, Urzainqui A, Bevan M, Martin C, Smeekens S, Tonelli C, Paz-Ares J, Weisshaar B (1998) Towards functional characterization of the members of the *R2R3-MYB* gene family from *Arabidopsis thaliana*. Plant J 16:263–276

Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, Ito J, Mimura T, Fukuda H, Demura T (2005) Transcription switches for protoxylem and metaxylem vessel formation. Genes Dev 19:1855–1860

Lauvergeat V, Lacomme C, Lacombe E, Lasserre E, Roby D, Grima-Pettenati J (2001) Two cinnamoyl-CoA reductase (CCR) genes from *Arabidopsis thaliana* are differentially expressed during development and in response to infection with pathogenic bacteria. Phytochemistry 57:1187–1195

Legay S, Lacombe E, Goicoechea M, Brière C, Séguin A, Mackay J, Grima-Pettenati J (2007) Molecular characterization of *EgMYB1*, a putative transcriptional repressor of the lignin biosynthetic pathway. Plant Sci 173:542–549

Li L, Popko JL, Zhang X-H, Osakabe K, Tsai C-J, Joshi CP, Chiang VL (1997) A novel multifunctional *O*-methyltransferase implicated in a dual methylation pathway associated with lignin biosynthesis in loblolly pine. Proc Natl Acad Sci USA 94:5461–5466

Li L, Popko JL, Umezawa T, Chiang VL (2000) 5-Hydroxyconiferyl aldehyde modulates enzymatic methylation for syringyl monolignol formation, a new view of monolignol biosynthesis in angiosperms. J Biol Chem 275:6537–6545

Li L, Cheng XF, Leshkevich J, Umezawa T, Harding SA, Chiang VL (2001) The last step of syringyl monolignol biosynthesis in angiosperms is regulated by a novel gene encoding sinapyl alcohol dehydrogenase. Plant Cell 13:1567–1585

Lu S, Sun Y-H, Shi R, Clark C, Li L, Chiang VL (2005) Novel and mechanical stress–responsive microRNAs in *Populus trichocarpa* that are absent from Arabidopsis. Plant Cell 17:2186–2203

Marita JM, Ralph J, Lapierre C, Jouanin L, Boerjan W (2001) NMR characterization of lignins from transgenic poplars with suppressed caffeic acid *O*-methyltransferase activity. J Chem Soc Perkin Trans 1:2939–2945

Marita JM, Ralph J, Hatfield RD, Guo D, Chen F, Dixon RA (2003) Structural and compositional modifications in lignin of transgenic alfalfa down-regulated in caffeic acid 3-*O*-methyltransferase and caffeoyl coenzyme A 3-*O*-methyltransferase. Phytochemistry 62:53–65

Matsui N, Chen F, Yasuda S, Fukushima K (2000) Conversion of guaiacyl to syringyl moieties on the cinnamyl alcohol

pathway during the biosynthesis of lignin in angiosperms. Planta 210:831–835

Meyermans H, Morreel K, Lapierre C, Pollet B, De Bruyn A, Busson R, Herdewijn P, Devreese B, Van Beeumen J, Marita JM, Ralph J, Chen C, Burggraeve B, Van Montagu M, Messens E, Boerjan W (2000) Modifications in lignin and accumulation of phenolic glucosides in poplar xylem upon down-regulation of caffeoyl-coenzyme A *O*-methyltransferase, an enzyme involved in lignin biosynthesis. J Biol Chem 275:36899–36909

Mitsuda N, Seki M, Shinozaki K, Ohme-Takagi M (2005) The NAC transcription factors NST1 and NST2 of *Arabidopsis* regulates secondary wall thickening and are required for anther dehiscence. Plant Cell 17:2993–3006

Mitsuda N, Iwase A, Yamamoto H, Yoshida M, Seki M, Shinozaki K, Ohme-Takagi M (2007) NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of *Arabidopsis*. Plant Cell 19:270–280

Moyle R, Moody J, Phillips L, Walter C, Wagner A (2002) Isolation and characterization of a *Pinus radiata* lignin biosynthesis-related *O*-methyltransferase promoter. Plant Cell Rep 20:1052–1060

Muzac I, Wang J, Anzellotti D, Zhang H, Ibrahim RK (2000) Functional expression of an *Arabidopsis* cDNA clone encoding a flavonol 3'-*O*-methyltransferase and characterization of the gene product. Arch Biochem Biophys 375:385–388

Nair RB, Xia Q, Kartha CJ, Kurylo E, Hirji RN, Datla R, Selvaraj G (2002) Arabidopsis CYP98A3 mediating aromatic 3-hydroxylation. Developmental regulation of the gene, and expression in yeast. Plant Physiol 130:210–220

Nakatsubo T, Li L, Hattori T, Lu S, Sakakibara N, Chiang VL, Shimada M, Suzuki S, Umezawa T (2007a) Roles of 5-hydroxyconiferylaldehyde and caffeoyl CoA *O*-methyltransferases in monolignol biosynthesis in *Carthamus tinctorius*. Cellulose Chem Technol 41:511–520

Nakatsubo T, Suzuki S, Hattori T, Umezawa T (2007b) A novel 5-hydroxyconiferaldehyde/5-hydroxyconiferyl alcohol *O*-methyltransferase for monolignol synthesis. In: Abstracts of the 25th annual meeting of the Japanese society for plant cell and molecular biology, Chiba University, Chiba, Japan, p 121, 8–9 August 2007

Nakatsubo T, Kitamura Y, Sakakibara N, Mizutani M, Hattori T, Sakurai N, Shibata D, Suzuki S, Umezawa T (2008) At5g54160 gene encodes *Arabidopsis thaliana* 5-hydroxyconiferaldehyde *O*-methyltransferase. J Wood Sci 54:312–317

Newman LJ, Perazza DE, Juda L, Campbell MM (2004) Involvement of the R2R3-MYB, *At*MYB61, in the ectopic lignification and dark-photomorphogenic components of the *det3* mutant phenotype. Plant J 37:239–250

Ni W, Paiva NL, Dixon RA (1994) Reduced lignin in transgenic plants containing a caffeic acid *O*-methyltransferase antisense gene. Transgenic Res 3:120–126

Niggeweg R, Michael AJ, Martin C (2004) Engineering plants with increased levels of the antioxidant chlorogenic acid. Nat Biotechnol 22:746–754

Olsen AN, Ernst HA, Leggio LL, Skriver K (2005) NAC transcription factors: structurally distinct, functionally diverse. Trends Plant Sci 10:79–87

Osakabe K, Tsao CC, Li L, Popko JL, Umezawa T, Carraway DT, Smeltzer RH, Joshi CP, Chiang VL (1999) Coniferyl aldehyde 5-hydroxylation and methylation direct syringyl lignin biosynthesis in angiosperms. Proc Natl Acad Sci USA 96:8955–8960

Parvathi K, Chen F, Guo D, Blount JW, Dixon RA (2001) Substrate preferences of *O*-methyltransferases in alfalfa suggest new pathways for 3-*O*-methylation of monolignols. Plant J 25:193–202

Patzlaff A, Newman LJ, Dubos C, Whetten RW, Smith C, McInnis S, Bevan MW, Sederoff RR, Campbell MM (2003a) Characterisation of *Pt*MYB1, an R2R3-MYB from pine xylem. Plant Mol Biol 53:597–608

Patzlaff A, McInnis S, Courtenay A, Surman C, Newman LJ, Smith C, Bevan MW, Mansfield S, Whetten RW, Sederoff RR, Campbell MM (2003b) Characterisation of a pine MYB that regulates lignification. Plant J 36:743–754

Preston J, Wheeler J, Heazlewood J, Li SF, Parish RW (2004) AtMYB32 is required for normal pollen development in *Arabidopsis thaliana*. Plant J 40:979–995

Raes J, Rohde A, Christensen JH, Van de Peer Y, Boerjan W (2003) Genome-wide characterization of the lignification toolbox in Arabidopsis. Plant Physiol 133:1051–1071

Ralph J, Lapierre C, Marita JM, Kim H, Lu F, Hatfield RD, Ralph S, Chapple C, Franke R, Hemm MR, Van Doorsselaere J, Sederoff RR, O'Malley DM, Scott JT, MacKay JJ, Yahiaoui N, Boudet A-M, Pean M, Pilate G, Jouanin L, Boerjan W (2001) Elucidation of new structures in lignins of CAD- and COMT-deficient plants by NMR. Phytochemistry 57:993–1003

Ralph J, Lundquist K, Brunow G, Lu F, Kim H, Schatz PF, Marita JM, Hatfield RD, Ralph SA, Christensen JH, Boerjan W (2004a) Lignins: natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids. Phytochem Rev 3:29–60

Ralph J, Bunzel M, Marita JM, Hatfield RD, Lu F, Kim H, Schatz PF, Grabber JH, Steinhart H (2004b) Peroxidase-dependent cross-linking reactions of *p*-hydroxycinnamates in plant cell walls. Phytochem Rev 3:79–96

Ralph J, Akiyama T, Kim H, Lu F, Schatz PF, Marita JM, Ralph SA, Reddy MSS, Chen F, Dixon RA (2006) Effects of coumarate 3-hydroxylase down-regulation on lignin structure. J Biol Chem 281:8843–8853

Rogers LA, Campbell MM (2004) The genetic control of lignin deposition during plant growth and development. New Phytol 164:17–30

Sakakibara N, Nakatsubo T, Suzuki S, Shibata D, Shimada M, Umezawa T (2007) Metabolic analysis of the cinnamate/monolignol pathway in *Carthamus tinctorius* seeds by a stable-isotope-dilution method. Org Biomol Chem 5: 802–815

Sarkanen KV (1971) Biogenensis of lignin precursors. In: Sarkanen KV, Ludwig CH (eds) Lignins. Wiley-Interscience, New York, pp 95–115

Schneider K, Hövel K, Witzel K, Hamberger B, Schomburg D, Kombrink E, Stuible H-P (2003) The substrate specificity-determining amino acid code of 4-coumarate: CoA ligase. Proc Natl Acad Sci USA 100:8601–8606

Schoch G, Goepfert S, Morant M, Hehn A, Meyer D, Ullmann P, Werck-Reichhart D (2001) CYP98A3 from *Arabidopsis thaliana* is a 3'-hydroxylase of phenolic esters, a

missing link in the phenylpropanoid pathway. J Biol Chem 276:36566–36574

Sewalt VJH, Ni W, Blount JW, Jung HG, Masoud SA, Howles PA, Lamb C, Dixon RA (1997a) Reduced lignin content and altered lignin composition in transgenic tobacco down-regulated in expression of L-phenylalanine ammonia-lyase or cinnamate 4-hydroxylase. Plant Physiol 115:41–50

Sewalt VJH, Ni W, Jung HG, Dixon RA (1997b) Lignin impact on fiber degradation: increased enzymatic digestibility of genetically engineered tobacco (*Nicotiana tabacum*) stems reduced in lignin content. J Agric Food Chem 45:1977–1983

Stracke R, Werber M, Weisshaar B (2001) The *R2R3-MYB* gene family in *Arabidopsis thaliana*. Curr Opin Plant Biol 4:447–456

Suzuki S, Umezawa T (2007) Biosynthesis of lignans and norlignans. J Wood Sci 53:273–284

Tamagnone L, Merida A, Parr A, Mackay S, Culianez-Macia FA, Roberts K, Martin C (1998) The AmMYB308 and AmMYB330 transcription factors from Antirrhinum regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco. Plant Cell 10:135–154

Tohge T, Nishiyama Y, Hirai MY, Yano M, Nakajima J-i, Awazuhara M, Inoue E, Takahashi H, Goodenowe DB, Kitayama M, Noji M, Yamazaki M, Saito K (2005) Functional genomics by integrated analysis of metabolome and transcriptome of Arabidopsis plants over-expressing an MYB transcription factor. Plant J 42:218–235

Tohge T, Yonekura-Sakakibara K, Niida R, Watanabe-Takahashi A, Saito K (2007) Phytochemical genomics in *Arabidopsis thaliana*: a case study for functional identification of flavonoid biosynthesis genes. Pure Appl Chem 79:811–823

Tsai C-J, Popko JL, Mielke MR, Hu W-J, Podila GK, Chiang VL (1998) Suppression of *O*-methyltransferase gene by homologous sense transgene in quaking aspen causes red-brown wood phenotypes. Plant Physiol 117:101–112

Umezawa T (2001) Biosynthesis of lignans and related phenylpropanoid compounds. Regul Plant Growth Dev 36:57–67

Umezawa T (2003) Diversity in lignan biosynthesis. Phytochem Rev 2:371–390

Umezawa T (2005) Biosynthesis of lignans, lignins, and norlignans. Kagaku To Seibutsu 43:461–467

van de Mortel JE, Schat H, Moerland PD, Ver Loren van, Themaat E, van der Ent S, Blankestijn H, Ghandilyan A, Tsiatsiani S, Aarts MGM (2008) Expression differences for genes involved in lignin, glutathione and sulphate metabolism in response to cadmium in *Arabidopsis thaliana* and the related Zn/Cd-hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ 31:301–324

Van Doorsselaere J, Baucher M, Chognot E, Chabbert B, Tollier M-T, Petit-Conil M, Leplé J-C, Pilate G, Cornu D, Monties B, Van Montagu M, Inzé D, Boerjan W, Jouanin L (1995) A novel lignin in poplar trees with a reduced caffeic acid/5-hydroxyferulic acid *O*-methyltransferase activity. Plant J 8:855–864

Vanholme R, Morreel K, Ralph J, Boerjan W (2008) Lignin engineering. Curr Opin Plant Biol 11:278–285

Wagner A, Ralph J, Akiyama T, Flint H, Phillips L, Torr K, Nanayakkara B, Kiri LT (2007) Exploring lignification in conifers by silencing hydroxycinnamoyl-CoA: shikimate hydroxycinnamoyltransferase in *Pinus radiata*. Proc Natl Acad Sci USA 104:11856–11861

Weng J-K, Li X, Bonawitz ND, Chapple C (2008a) Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. Curr Opin Biotech 19:166–172

Weng J-K, Li X, Stout J, Chapple C (2008b) Independent origins of syringyl lignin in vascular plants. Proc Natl Acad Sci USA 105:7887–7892

Yang C, Xu Z, Song J, Conner K, Barrena GV, Wilson ZA (2007) *Arabidopsis MYB26/MALE STERILE35* regulates secondary thickening in the endothecium and is essential for anther dehiscence. Plant Cell 19:534–548

Ye Z-H, Kneusel RE, Matern U, Varner JE (1994) An alternative methylation pathway in lignin biosynthesis in *Zinnia*. Plant Cell 6:1427–1439

Ye Z-H, Zhong R, Morrison WH III, Himmelsbach DS (2001) Caffeoyl coenzyme A *O*-methyltransferase and lignin biosynthesis. Phytochemistry 57:1177–1185

Zhang H, Wang J, Goodman HM (1997) An *Arabidopsis* gene encoding a putative 14-3-3-interacting protein, caffeic acid/5-hydroxyferulic acid *O*-methyltransferase. Biochem Biophys Acta 1353:199–202

Zhang B, Pan X, Cobb GP, Anderson TA (2006a) Plant microRNA: a small regulatory molecule with big impact. Developmental Biol 289:3–16

Zhang K, Qian Q, Huang Z, Wang Y, Li M, Hong L, Zeng D, Gu M, Chu C, Cheng Z (2006b) *GOLD HULL AND INTERNODE2* encodes a primarily multifunctional cinnamyl-alcohol dehydrogenase in rice. Plant Physiol 140:972–983

Zhong R, Ye Z-H (2007) Regulation of cell wall biosynthesis. Curr Opin Plant Biol 10:564–572

Zhong R, Morrison WH III, Negrel J, Ye Z-H (1998) Dual methylation pathways in lignin biosynthesis. Plant Cell 10:2033–2045

Zhong R, Morrison WH III, Himmelsbach DS, Poole FL II, Ye Z-H (2000) Essential role of caffeoyl coenzyme A *O*-methyltransferase in lignin biosynthesis in woody poplar plants. Plant Physiol 124:563–577

Zhong R, Demura T, Ye Z-H (2006) SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of *Arabidopsis*. Plant Cell 18: 3158–3170

Zhong R, Richardson EA, Ye Z-H (2007a) The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in *Arabidopsis*. Plant Cell 19:2776–2792

Zhong R, Richardson EA, Ye Z-H (2007b) Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of *Arabidopsis*. Planta 225:1603–1611

Zhong R, Lee C, Zhou J, McCarthy RL, Ye Z-H (2008) A battery of transcription factors involved in the regulation

of secondary cell wall biosynthesis in *Arabidopsis*. Plant Cell 20:2763–2782

Zhou J, Lee C, Zhong R, Ye Z-H (2009) MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in *Arabidopsis*. Plant Cell 21:248–266

Zubieta C, Kota P, Ferrer J-L, Dixon RA, Noel JP (2002) Structural basis for the modulation of lignin monomer methylation by caffeic acid/5-hydroxyferulic acid 3/5-*O*-methyltransferase. Plant Cell 14:1265–1277